Nicotinamide

Catalog # 07154 100 grams per bottle

PRODUCT DESCRIPTION:

Nicotinamide ($C_6H_6N_2O$)
Molecular Weight: 122.1 g/mol

This product has been developed for use with the ES-Cult™ Basal Medium-A and N2 Supplement-A. It is used to improve the yield of pancreatic endocrine cells during the final stages of ES *in vitro* differentiation into pancreatic islet-like insulin secreting cells.

This product has been pre-screened for use with other reagents of the ES-Cult™ product line for optimal performance for the *in vitro* pancreatic differentiation of murine ES cells.

STABILITY / STORAGE:

Store at room temperature.

DIRECTIONS FOR USE:

Prepare a 1M solution of nicotinamide by dissolving 0.122g of nicotinamide per mL of sterile water. This provides a 100X stock solution and should be added to the ES-Cult™ Basal Medium-A at a final concentration of 10 mM. This stock solution is stable for one year from date of preparation. For further information refer to the Technical Manual "*In Vitro* Differentiation of Mouse Embryonic Stem Cells into Insulin Secreting Pancreatic Islet-like Clusters" on our website at www.stemcell.com.

THIS REAGENT IS FOR LABORATORY USE ONLY.
IT IS NOT TO BE ADMINISTERED TO HUMANS.

REFERENCES:

1. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, and McKay R. Differentiation of Embryonic Stem Cells to Insulin-Secreting Structures Similar to Pancreatic Islets. Science 2001:292:1389.

2. Otonkoski T, Beattie GM, Mally MI, Ricordi C, and Hayek A. Nicotinamide is a potent inducer of endocrine differentiation in cultured human fetal pancreatic cells. J Clin Invest. 1993:92:1459

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Protocol for the *In Vitro* Differentiation of Mouse Embryonic Stem Cells into Insulin Secreting Pancreatic Islet-like Clusters

This protocol is designed to differentiate mouse embryonic stem (ES) cells to insulin secreting pancreatic islet-like clusters using culture conditions described by McKay et al (1). This protocol was optimized using the R1 ES cell line. Results obtained will depend upon the ES cell line, the maintenance conditions, and the supplements and growth factors used. Results may also vary if non-ES-Cult™ reagents are substituted within the protocol. A more detailed Technical Manual is available on our website at www.stemcell.com.

Step 1: Passage of ES cells onto gelatinized dishes (Pre-differentiation)

ES Maintenance Media (final concentration): 15% FCS (#06902/06952), 1 mM Sodium Pyruvate (#07000), 2mM L-glutamine (#07100), 0.1 mM MEM Non-Essential Amino Acids (#07600), 20 ng/mL murine Leukemia Inhibitory Factor (#02740), 100µM MTG (Sigma #M-6145), 100 U/mL Penicillin G and 100µg/mL Streptomycin (#07500) and DMEM High Glucose (#36250). Store at 4°C for up to 2 weeks.

1.1 Coat appropriate number of tissue culture dishes with gelatin (#07903), by adding enough gelatin to cover the surface of the dish completely. Incubate for 20 minutes at room temperature. Aspirate and dry dishes in hood.

1.2 Harvest ES cells: Remove media and rinse cells once with PBS (#37350). Add Trypsin-EDTA (#07901) to completely cover dish and incubate for 3-5 minutes at 37°C. Transfer cell and trypsin solution into a tube containing 8 mL of DMEM +10% FCS and spin at 1200 rpm for 7 minutes. Aspirate medium and resuspend pellet in 2 mL of ES Maintenance Media. **Pipette up and down several times to ensure a single cell suspension.** Perform a 1/10 dilution or plate 1-1.5 $x10^6$ cells/100 mm gelatinized dish containing 8 mL of medium and incubate at 37°C for two days.

Step 2: Formation of embryoid bodies (EBs) in suspension culture

ES Differentiation Medium (final concentration): 15% FCS (#06905), 0.1 mM MEM Non-Essential Amino Acids (#07600), 2 mM L-glutamine (#07100), 981 µM MTG (Sigma #M-6145) and DMEM High Glucose (#36250). Store at 4°C for up to 2 weeks. Use of Ultra-Low Adherent Dishes (#27145) reduces the attachment of EBs to the surface of the dish.

2.1 Add ES Differentiation Medium to the Ultra-Low Adherent Dishes (3 mL/well, 6 wells per plate) and place at 37°C for 15-30 minutes to rehydrate the surface.

2.2 Harvest ES cells as detailed in Step 1.2.

2.3 Plate 5 $x10^5$ cells/well and incubate at 37°C for two days.

2.4 Perform a media change on the second day by collecting EBs from one well into a 14mL polystyrene tube. Let EBs settle to the bottom of the tube for ~3-5 minutes (there is no need to centrifuge). Remove media, add 3mL of ES Differentiation Medium and transfer back to original well.

2.5 Incubate at 37°C for an additional two days.

Step 3: Enrichment of nestin positive cells

Serum-Free ITS-A Medium (final concentration): ES-Cult™ Basal Medium-A (#05801) containing 1X ITS Supplement-A (#07151). Store at 4°C for up to 2 weeks.

3.1 On the fourth day, transfer EBs to a 14mL tube and let stand to allow EBs to settle.

3.2 Remove media, add 3mL of Serum-Free ITS-A Medium and transfer to one well of a 6 well **tissue culture dish**.

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Grow for six days, with a media change every second day.

Prior to Step 4, prepare cultureware for expansion and differentiation of cells to insulin secreting pancreatic islet-like structures as follows:

- For immunohistochemistry, cells are grown on coverslips (Fisher Scientific #12-545-82) that can be stained and mounted onto a slide at the end of the protocol. Coverslips should be soaked in ethanol for 5-15 minutes, cleaned of any debris with a tissue and then autoclaved. Using sterilized tweezers, insert one coverslip per well of a 24 well plate. Insulin secretion can also be quantified (use 6 wells of a 24 well plate) using an ELISA kit (ALPCO Diagnostics #008-10-1124-01).
- Coverslips (for staining) and/or individual wells (for ELISA) of a 24 well plate are coated with poly-L-ornithine (Sigma #P3655), used at a final concentration of 15 µg/mL diluted in PBS (#37350). Add 1 mL/well of diluted poly-L-ornithine solution and incubate at 37°C for one hour or overnight at 4°C. Rinse three times with PBS (incubate for 5 minutes at room temperature/wash)

Step 4: Expansion of enriched cells

Pancreatic Proliferation Medium (final concentration): ES-Cult™ Basal Medium-A (#05801), 1X N2-Supplement-A (#07152), 1X B27 Supplements (#07153), 25 ng/mL bFGF recombinant human (#02634). Store at 4°C for up to 2 weeks.

4.1 Remove all PBS and add 1 mL/well of Pancreatic Proliferation Medium.

4.2 Harvest cells: Remove media, add 1 mL/well of Trypsin-EDTA (#07901) and incubate at 37°C for 3 minutes. Add 3 mL of DMEM+10% FBS to each well and transfer cell and trypsin solution to a 14 mL tube. At this stage, there is a tendency for the cells to form clumps, making it difficult to obtain a single cell suspension. Therefore, let cell solution stand for a few minutes (< 3 minutes) so clumps settle to the bottom of tube. **DO NOT SPIN DOWN**! The supernatant is then removed from the tube and centrifuged at 1200 rpm for 5-7 minutes. Cells are resuspended in 1 mL of Pancreatic Proliferation Medium and counted.

4.3 Plate $5x10^5$ cells/well (poly-L-ornithine coated well or coated coverslip) of a 24 well plate in 1 mL of media. Media is changed every two days for six days.

Step 5: Differentiation to insulin secreting pancreatic islet-like clusters

Pancreatic Differentiation Medium (final concentration): ES-Cult™ Basal Medium-A (#05801), 1X N2 Supplement-A (#07152), 1X B27 Supplements (#07153) and 10 mM Nicotinamide (#07154). Store at 4°C for up to 2 weeks.

- On the sixth day, remove media from wells and add 1 mL/well of Pancreatic Differentiation Medium. Grow for 6 days, changing media every second day.

Cells are now ready for immunohistochemistry staining and/or for ELISA. Protocols for immunohistochemistry staining and preparation of cells for ELISA are detailed in the Technical Manual available on our website at www.stemcell.com.

Reference:

Lumelsky, N., Blondel, O., Laeng, P., Velasco, I., Ravin, R., and McKay, R. (2001). Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science. 292:1389-1394.